New High Polarity bis (Cyanopropyl) Siloxane Stationary Phase for GC Resolution of Positional and Geometric Isomers of Fatty Acid Methyl Esters.

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Abstract

Very high percentage cyanopropyl (80-100%) containing polysiloxane stationary phases are used for the capillary GC analysis of fatty acid methyl esters (FAME) when characterization of geometric and positional isomers of fats and oils is required. The high polarity of these stationary phases is necessary to better resolve the potentially numerous cis/trans FAMEs in these samples.

High cyanopropyl content polysiloxanes have always presented problems with film stability both during initial coating of the capillary column and later, with use at higher temperatures as the viscosity of the polymer decreases. This is aggravated by the fact that extremely long columns of at least 100 meters are typically required to generate the separating power needed to adequately resolve positional isomers. To address these issues, an approximately 88% cyanopropyl polysiloxane stationary phase is stabilized by the incorporation arylene bridges into the polymer backbone and otherwise "tuned" by structural modifications to behave properly as a capillary GC stationary phase. This column has distinct advantages in temperature limits and separation quality relative to other columns commonly used for FAMES applications. The elution of sixty-seven individual FAMEs have been characterized on HP-88, and an algorithm has been developed to allow the prediction of elution order changes with chromatographic temperature changes.

Characterizing Elution of 67 FAMEs

Analysis of FAMEs (Fatty Acid Methyl Ester) found in edible oils, processed foods, conventional foods, and biological samples is now better enabled by the rugged characterization of over 67 important saturated, unsaturated, and cis and trans-FAMES on Agilent's HP-88 GC Column (Table 1). This represents the most extensive characterization of FAMES published by any column manufacturer to date, and allows analysts to more confidently confirm the identification of specific FAMES of interest in new or unusual samples, or to switch from another manufacturer's previous generation column to Agilent's HP-88 column.

HP-88 is a more stable, more efficient arylene cyanopropyl stationary phase version compared to previous generation non-arylene cyanopropyl columns used for this analysis. Because of it's higher upper temperature limit, analysts using HP-88 should enjoy longer lifetime and better resolution of FAMEs than conventional cyanopropyl columns.

No one column can resolve all possible FAME permutations of, say, a highly polyunsaturated edible oil which has been partially hydrogenated. However, HP-88 has a feature that can allow a more complete characterization: temperature dependent selectivity[1]. Temperature dependent selectivity means that you can, just by changing the average chromatographic temperature the compounds experience, improve the separation order of some compounds of interest. Practically, this means that more FAMEs can be identified in very complex samples just by changing the temperature conditions (see Table 1 and explanation to follow). The temperature change required to get meaningful selectivity changes however is a large (30ºC), so analysts do not have to worry about elution order changes over minor deviations in temperature that normally result from routine column maintenance or slight changes in column flow for temperature programmed runs.

Temperature dependant selectivity is sometimes counter intuitive. Normally, one would think that to improve a separation you simply lower the temperature. It is not always the case. To aid in maximizing the resolution between any two FAMEs Agilent provides an Excel tool that enables the analyst to determine whether to increase or decrease the average chromatographic temperature in order to improve the resolution, and also will indicate at what temperature the two FAMEs of interest will co-elute. This tool can be downloaded from the Agilent web site. Visit www.agilent.com/chem, click Columns & Accessories then All Column Phases then HP-88 for a downloadable spreadsheet that will allow you to determine the elution order of the listed FAMEs at a given temperature and unretained peak time."

[1] *J. High Resol. Chromatography. 14:745-750 (1991)*

Table 1. Elution order of 67 FAMEs on HP-88 at 155ºC and 180ºC. Elution order reversals are in bold.

	Tm	5.67
Isothermal Temp (C)	155	180
CHAIN	RT	RT
C08:0	6.47	6.03
C11:0	8.13	6.67
C11:1	9.11	7.07
C12:0	9.28	7.09
C12:1	10.48	7.53
C13:0	10.66	7.53
C13:1	12.49	8.21
C14:0	12.78	8.24
C15:1n5 - trans	13.12	8.35
C14:1n5 - trans	14.01	8.66
C14:1	14.73	8.92
C15:0	15.48	9.03
C15:1n5	18.19	9.96
C15:1n1	18.65	10.05
C16:0	19.24	10.10
C16:1 - trans	21.66	11.03
C16:1	22.69	11.40
C17:0	25.15	12.05
C17:1n7 - trans	27.83	12.91
C17:1	29.16	13.39
C18:0	32.41	14.00
C16:3	34.16	**15.11**
C18:1n12 - trans	35.21	**14.89**
C18:1n9 - trans	35.38	14.97
C18:1n7 - trans	35.96	15.10
C18:1n12	36.51	15.22
C18:1n9	36.89	15.35
C18:1n7	37.78	15.57
C18:2n6 - trans	41.62	16.66
C18:2n6	45.30	18.56
C19:1n12 - trans	45.66	18.22
C19:1n9 - trans	48.25	18.64
C18:3n6	53.08	**20.70**
C20:0	55.85	**20.64**
C18:3n3	58.99	22.28
C19:2n6	59.81	**22.48**
C20:1n9 - trans	61.02	**22.26**
C18:2-d10 trans,d12 trans (conjugated)	62.64	23.30
C18:2- d9 cis, d11 trans (conjugated)	63.35	**23.36**
C20:1n9	63.45	**23.09**
C18:2-d10 cis,d12 trans (conjugated)	64.63	23.88
C18:2- d10 trans, d12 cis (conjugated)	65.50	24.01
C18:4n3	68.23	**25.37**
C21:0	75.55	**25.14**
C20:2n6	79.30	27.55
C20:3n9	82.90	**28.86**
C21:1n9	85.70	**28.24**
C20:3n6	91.81	31.24
C22:0	100.24	31.50
C20:4n6	101.91	**34.25**
C20:3n3	103.03	**33.96**
C21:2n6	106.08	**34.43**
C22:1n9 - trans	110.80	**33.84**
C22:1n9	111.68	35.61
C20:4n3	119.89	38.74
C20:5n3 (DPA)	131.26	**42.94**
C22:2n6	135.27	**42.61**
C23:1n9	143.19	44.19
C22:3n6	176.01	**53.19**
C22:4n6	178.91	**54.93**
C24:0	182.04	**50.00**
C22:5n6	195.05	**59.45**
C24:1n9	197.65	**56.34**
C22:5n3 (EPA)	233.41	69.01
C22:6n3 (DHA)	255.42	75.26
C24:5n3	409.85	110.29
C24:6n3	467.70	125.63

Elution orders and resolutions can change with a change in chromatographic temperature. This is not "new news." If you look hard enough, you can find a pair of compounds that reverse elution orders on any capillary GC column. What is less commonly understood and counter intuitive is that resolutions can be improved by increasing chromatographic temperature. The practical benefit of this is that for a complex sample that has co-elution at one temperature, it is possible to achieve complete resolution of all FAMEs simply by changing the temperature and using data from both runs to more fully characterize the sample, or simply modifying the current temperatur program.

Example: Looking at the table, a run at 155ºC gives the elution order of 18:2n6 (45.30 min) followed by C19:1n12-trans (45.66 min). If there is very large amount of 18:2n6 present and a small amount of C19:1n12-trans, the 18:2n6 peak will "front" and the C19:1n12-trans peak will be lost in the fronting. By RAISING the temperature to 180ºC, the C19:1n12-trans peak will actually elute before the 18:2n6 peak, and resolution will be regained.

How can the direction of elution order change be predicted?

Temperature dependent selectivity can predicted by characterizing the elution of different FAMEs at multiple temperatures (See Figure 1), and then plotting curves according to the following equation:

-T (Kelvin) x ln (k) vs Elution Temperature,

where k = (Retention Time of FAME – Unretained Peak Time)/Unretained peak time

This allows a linear curve to be fit of y = mx+b.

It is the differing slopes (m) that indicate whether or not two given compounds will potentially change resolution or even elution order by varying the chromatographic temperature. They also indicate which direction in temperature you have to go, increased or decreased, relative to where you are in order to improve resolution. The following graph demonstrates the previous example.

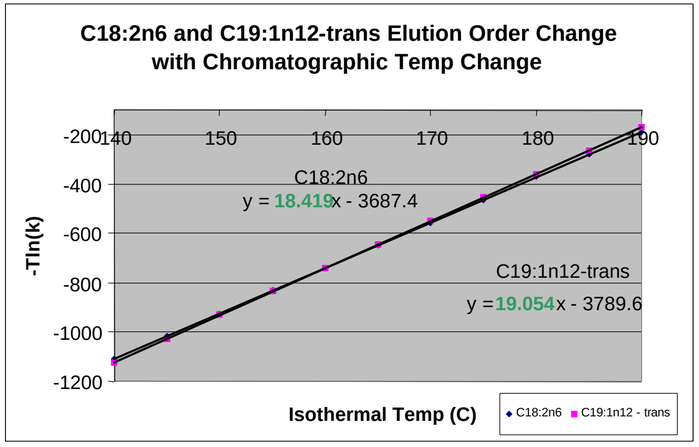

Figure 1. –Tln(k) v Chromatographic Temperature curve for C18:2n6 and C19:1n12-trans. These curve fit equations for each allow the prediction of elution order at any given temperature and the exact co-elution temperature. Additionally, one can predict retention times if the temperature and un-retained peak times are known.

While difficult to see graphically, note the two different slopes of the plots (in green) …this indicates the possibility for an elution order change by going higher or lower in temperature. By setting the the equations equal to each other and solving for x, one can also determine from the equations describing the two curves the temperature at which these two compounds exactly co-elute.

19:1n12-trans = 18:2n6

19.054x - 3789.6 = 18.419x - 3687.4

x (coelution temp) = 160.95ºC

If we wanted the 19:1n12-trans to elute before the 18:2n6, we would have to go up in chromatographic temperature from 160.95C. Referring to Table 1. retention times highlighted in green we note elution order reversal is achieved.

Conclusion

The following figures are a number of edible oil and fish oil chromatograms on HP-88, demonstrating that HP-88 is the most comprehensively characterized commercially high cyanopropyl column for FAMEs analysis available today. No other manufacturer has characterized and published the temperature dependent selectivity of their phase. As such, with HP-88, the analyst has much greater flexibility in achieving resolution between co-eluting pairs simply by changing the chromatographic temperature, and greater confidence in the identification of their FAMEs under a variety of chromatographic conditions. Combine this with the HP-88's higher upper temperature limit and you have a flexible and robust solution for identifying your FAMEs of interest.

Acknowledgments

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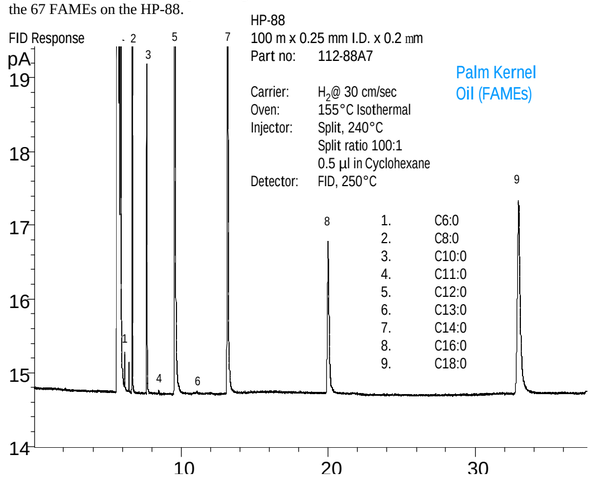
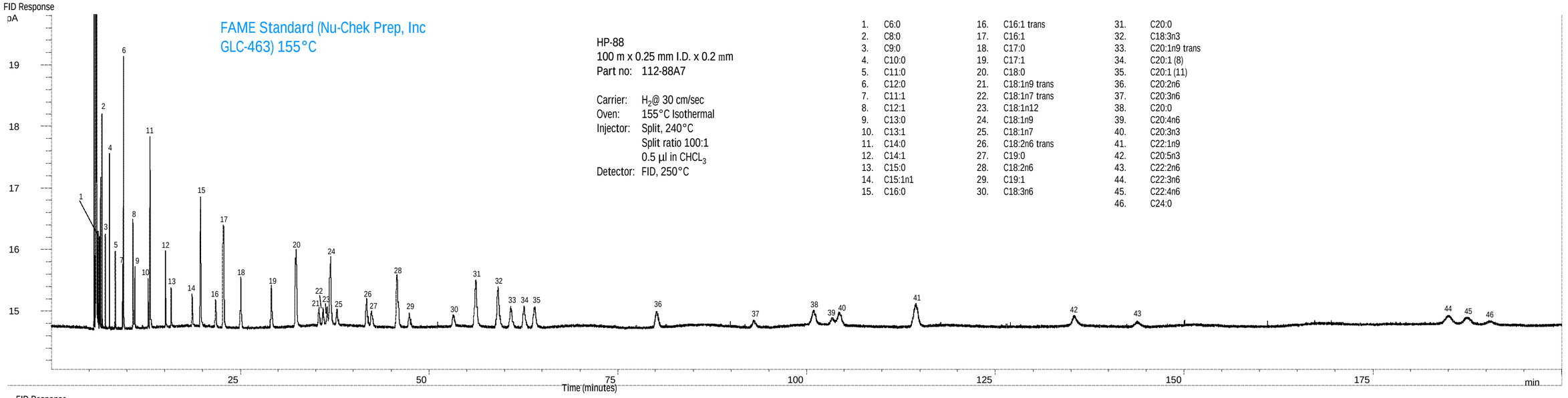
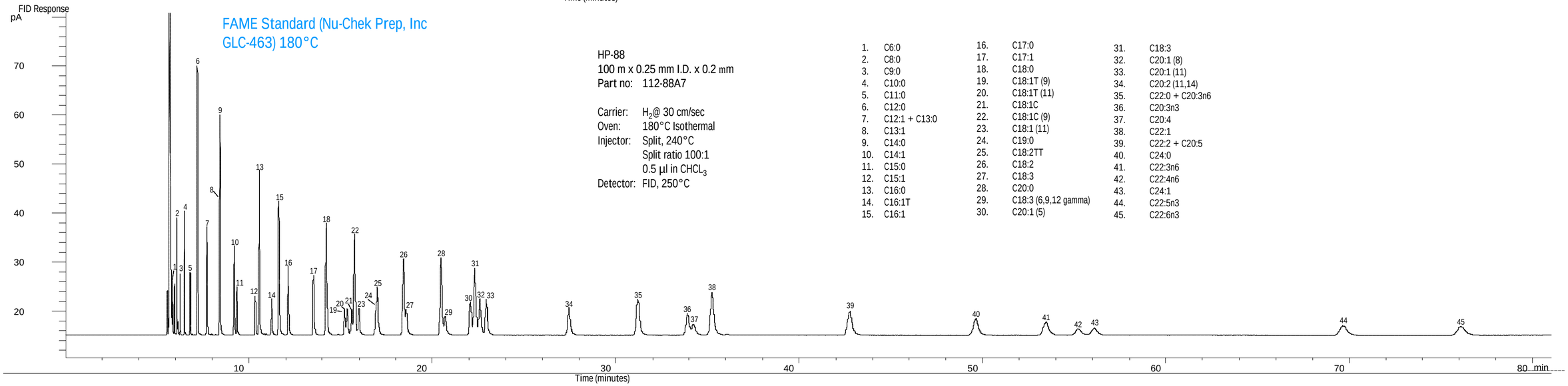



FID Response
pA
FAME Standard (Nu-Chek Prep, Inc
GLC-463) 155°C
HP-88
100 m x 0.25 mm I.D. x 0.2 mm
Part no: 112-88A7
Carrier: H2@ 30 cm/sec
Oven: 155°C Isothermal
Injector: Split, 240°C
Split ratio 100:1
0.5 µl in CHCL3
Detector: FID, 250°C
1. C6:0
2. C8:0
3. C9:0
4. C10:0
5. C11:0
6. C12:0
7. C11:1
8. C12:1
9. C13:0
10. C13:1
11. C14:0
12. C14:1
13. C15:0
14. C15:1n1
15. C16:0
16. C16:1 trans
17. C16:1
18. C17:0
19. C17:1
20. C18:0
21. C18:1n9 trans
22. C18:1n7 trans
23. C18:1n12
24. C18:1n9
25. C18:1n7
26. C18:2n6 trans
27. C19:0
28. C18:2n6
29. C19:1
30. C18:3n6
31. C20:0
32. C18:3n3
33. C20:1n9 trans
34. C20:1 (8)
35. C20:1 (11)
36. C20:2n6
37. C20:3n6
38. C20:0
39. C20:4n6
40. C20:3n3
41. C22:1n9
42. C20:5n3
43. C22:2n6
44. C22:3n6
45. C22:4n6
46. C24:0
Time (minutes)
min


FID Response
pA
FAME Standard (Nu-Chek Prep, Inc
GLC-463) 180°C
HP-88
100 m x 0.25 mm I.D. x 0.2 mm
Part no: 112-88A7
Carrier: H2@ 30 cm/sec
Oven: 180°C Isothermal
Injector: Split, 240°C
Split ratio 100:1
0.5 µl in CHCL3
Detector: FID, 250°C
1. C6:0
2. C8:0
3. C9:0
4. C10:0
5. C11:0
6. C12:0
7. C12:1 + C13:0
8. C13:1
9. C14:0
10. C14:1
11. C15:0
12. C15:1
13. C16:0
14. C16:1T
15. C16:1
16. C17:0
17. C17:1
18. C18:0
19. C18:1T (9)
20. C18:1T (11)
21. C18:1C
22. C18:1C (9)
23. C18:1 (11)
24. C19:0
25. C18:2TT
26. C18:2
27. C18:3
28. C20:0
29. C18:3 (6,9,12 gamma)
30. C20:1 (5)
31. C18:3
32. C20:1 (8)
33. C20:1 (11)
34. C20:2 (11,14)
35. C22:0 + C20:3n6
36. C20:3n3
37. C20:4
38. C22:1
39. C22:2 + C20:5
40. C24:0
41. C22:3n6
42. C22:4n6
43. C24:1
44. C22:5n3
45. C22:6n3
Time (minutes)
min

Flax Seed Oil Dietary Supplement (FAMEs)

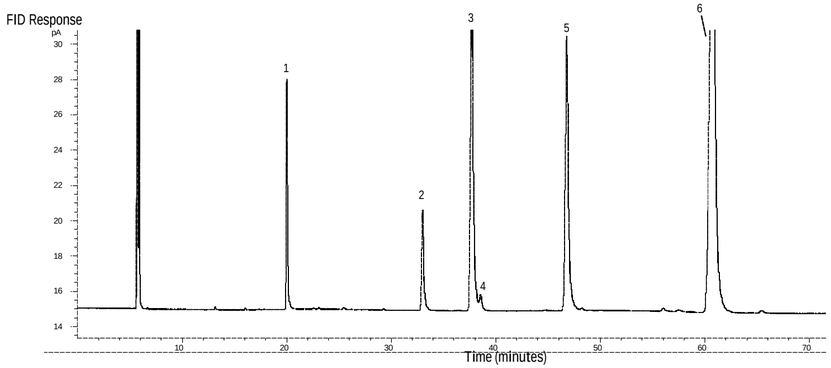
HP-88
100 m x 0.25 mm I.D. x 0.2 mm
Part no: 112-88A7

Carrier: H_2@ 30 cm/sec
Oven: 155°C Isothermal
Injector: Split, 240°C
Split ratio 100:1
0.5 µl in Cyclohexane
Detector: FID, 250°C

FID Response

Compound I.D.
(approximate Weight per 1000mg capsule)

1. C16:0 (5%)
2. C18:0 (3%)
3. C18:1n9 (17%)
4. C18:1n7 (9.5%)
5. C18:2n6 (15%)
6. C18:3n3 (52%)



Crude Safflower Oil (FAMEs)

HP-88
100 m x 0.25 mm I.D. x 0.2 mm
Part no: 112-88A7

Carrier: H_2@ 30 cm/sec
Oven: 180°C Isothermal
Injector: Split, 240°C
Split ratio 100:1
0.5 µl in Cyclohexane
Detector: FID, 250°C

1. C14:0
2. C14:1
3. C16:0
4. C16:1t
5. C16:1
6. C17:0
7. C17:1
8. C18:0
9. C18:1n9 trans
10. C18:1n9
11. C18:1n7
12. C22:0
13. C20:1n9 trans
14. C18:3n3
15. C20:1n9
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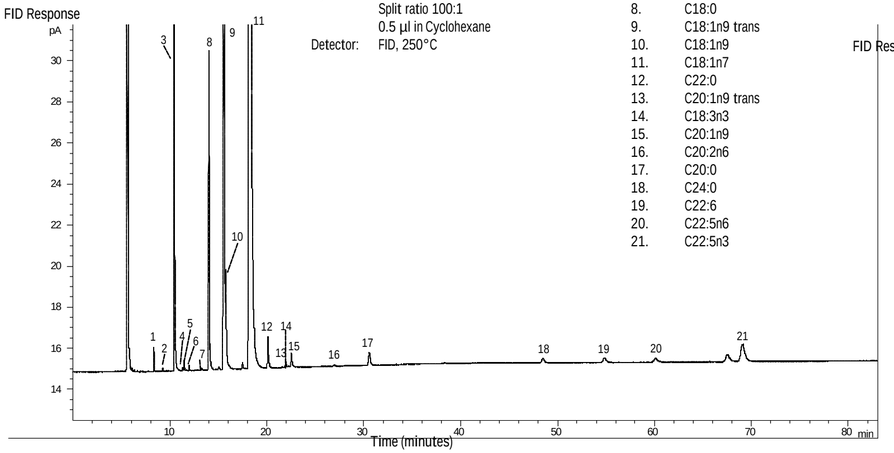
17. C20:0
18. C24:0
19. C22:6
20. C22:5n6
21. C22:5n3

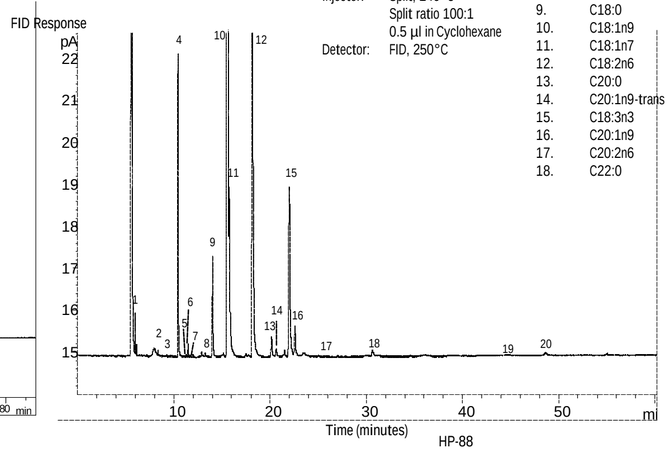


Canola (rapeseed) Oil (FAMEs)

HP-88
100 m x 0.25 mm I.D. x 0.2 mm
Part no: 112-88A7

Carrier: H_2@ 30 cm/sec
Oven: 180°C Isothermal
Injector: Split, 240°C
Split ratio 100:1
0.5 µl in Cyclohexane
Detector: FID, 250°C

1. C8:0
2. C14:0
3. C14:1
4. C16:0
5. C16:1-trans
6. C16:1
7. C17:0
8. C17:1
9. C18:0
10. C18:1n9
11. C18:1n7
12. C18:2n6
13. C20:0
14. C20:1n9-trans
15. C18:3n3
16. C20:1n9
17. C20:2n6
18. C22:0

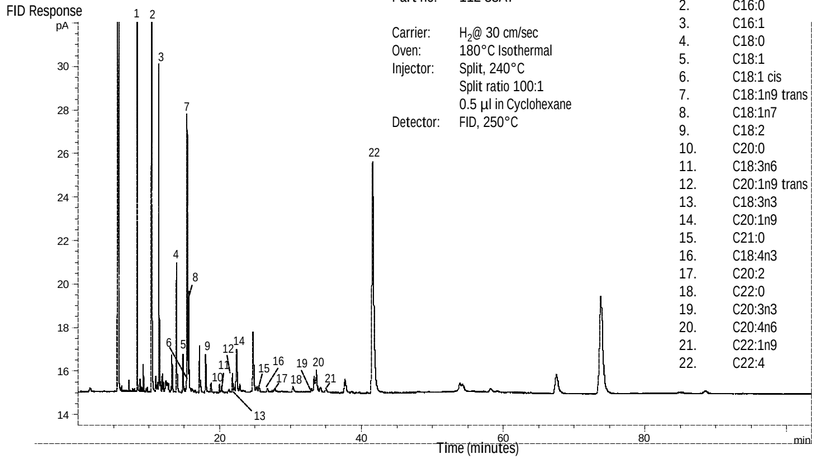


Fish Oil Dietary Supplement (FAMEs)

HP-88
100 m x 0.25 mm I.D. x 0.2 mm
Part no: 112-88A7

Carrier: H_2@ 30 cm/sec
Oven: 180°C Isothermal
Injector: Split, 240°C
Split ratio 100:1
0.5 µl in Cyclohexane
Detector: FID, 250°C

1. C14:0
2. C16:0
3. C16:1
4. C18:0
5. C18:1
6. C18:1 cis
7. C18:1n9 trans
8. C18:1n7
9. C18:2
10. C20:0
11. C18:3n6
12. C20:1n9 trans
13. C18:3n3
14. C20:1n9
15. C21:0
16. C18:4n3
17. C20:2
18. C22:0
19. C20:3n3
20. C20:4n6
21. C22:1n9
22. C22:4

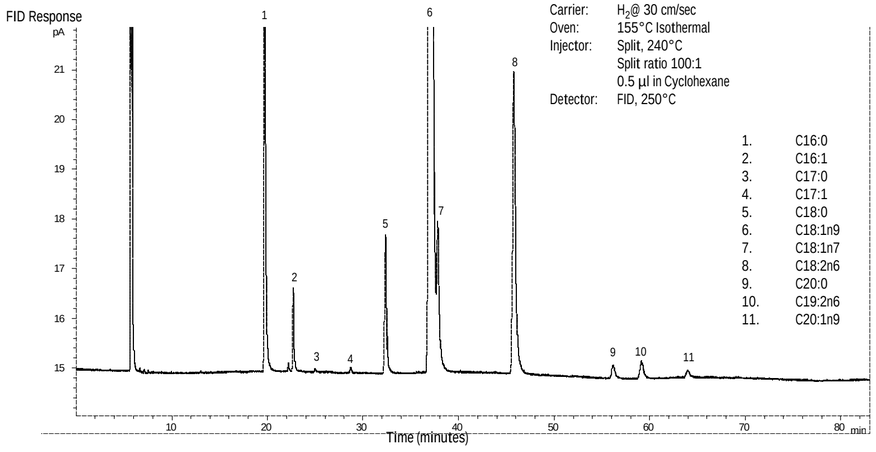


Olive Oil (FAMEs)

HP-88
100 m x 0.25 mm I.D. x 0.2 mm
Part no: 112-88A7

Carrier: H_2@ 30 cm/sec
Oven: 155°C Isothermal
Injector: Split, 240°C
Split ratio 100:1
0.5 µl in Cyclohexane
Detector: FID, 250°C

1. C16:0
2. C16:1
3. C17:0
4. C17:1
5. C18:0
6. C18:1n9
7. C18:1n7
8. C18:2n6
9. C20:0
10. C19:2n6
11. C20:1n9

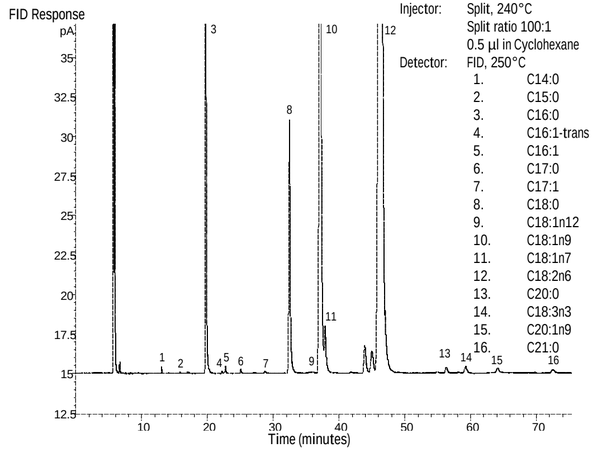


Grape Seed Oil (FAMEs)

HP-88
100 m x 0.25 mm I.D. x 0.2 mm
Part no: 112-88A7

Carrier: H_2@ 30 cm/sec
Oven: 155°C Isothermal
Injector: Split, 240°C
Split ratio 100:1
0.5 µl in Cyclohexane
Detector: FID, 250°C

1. C14:0
2. C15:0
3. C16:0
4. C16:1-trans
5. C16:1
6. C17:0
7. C17:1
8. C18:0
9. C18:1n12
10. C18:1n9
11. C18:1n7
12. C18:2n6
13. C20:0
14. C18:3n3
15. C20:1n9
16. C21:0



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